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(54) Title: COMPOSITION AND METHOD FOR TREATING CANCERS CHARACTERIZED BY OVER-EXPRESSION OF THE C-FMS PROTO-ONCOGENE (57) Abstract A composition and method for treating cancers characterized by over-expression of the <i>c-fms</i> proto-oncogene/M-CSF receptor protein are provided. The composition involves an M-CSF polypeptide cross-linked to a cytotoxic agent capable of crossing into the cytoplasm of the cell bearing the receptor and killing the cell.		

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COMPOSITION AND METHOD FOR TREATING CANCERS  
CHARACTERIZED BY OVER-EXPRESSION OF THE C-FMS PROTO-ONCOGENE

The present invention refers generally to the treatment of a variety of cancers characterized by the over-expression of the protein receptor, c-fms. More specifically, the invention refers to a composition for such treatment including the M-CSF polypeptide linked to a cytotoxic agent.

BACKGROUND OF THE INVENTION

A variety of oncogenes have been associated with specific cancers. The oncogene fms has come under recent scrutiny ~~as being related to breast, lung, pancreatic,~~ ovarian, renal, and possibly other carcinomas, including acute myelocytic leukemia (AML). See, e.g., D. J. Slamon et al, Science, 224:256-262 (1984); C. Walker et al, Proc. Natl. Acad. Sci., USA, :1804-1808 (April 1987). See also, J. H. Ohyashiki et al, Cancer Genet. Cytogenet., 25:341-350 (1987); H. D. Preisler et al, Cancer Research, 47:874-880 (Feb. 1987); C. W. Rettenmier et al, J. Cell. Biochem., 33:109-115 (1987); and R. Sacca et al, Proc. Natl. Acad. Sci. USA, 82:3331-3335 (1986). The product of the c-fms proto-oncogene is believed to be related to, and possibly identical with, a receptor of macrophage colony-stimulating factor (M-CSF). See, e.g., C. J. Sherr et al, Cell, 41:665-676 (1985);

There remains a need in the treatment of such cancers for therapeutic products capable of destroying the carcinoma cells without severely adversely affecting the patient otherwise.

BRIEF DESCRIPTION OF THE INVENTION

As one aspect of the invention there is provided a composition for treating cancers which are characterized by high level expression of the c-fms proto-oncogene/M-CSF

receptor gene. The composition includes M-CSF polypeptide (or the active fragment thereof) crosslinked to a cytotoxic agent, which is capable of crossing the membrane of the cell bearing the c-fms gene product/M-CSF receptor and acting in the cytoplasm to destroy the cell. Preferred cytotoxic agents include A and B chain toxins, A chain toxins and genetically engineered toxins.

In a further embodiment the composition may comprise a monoclonal antibody (or a portion thereof) to c-fms gene product/M-CSF receptor conjugated to a cytotoxic agent. This monoclonal moiety recognizes and binds to the c-fms gene product/M-CSF receptor. Antibody conjugates for the delivery of compounds to target sites and methods for preparing the same are known in the art. See e.g. U.S. Patent 4,671,958.

~~Still a further~~ aspect of the invention involves a method for making the M-CSF/cytotoxic agent composition. The M-CSF and toxin may be linked by employing one or more heterofunctional or bifunctional protein cross linkers or by genetic fusion. The bifunctional cross-linkers are chosen to ensure that the M-CSF/toxin composition is stable while the composition is homing to the target cell. At the same time the crosslinker has to permit the release of the toxin portion after the M-CSF/toxin composition has entered the cell. See, e.g. Molecular Action of Toxins and Viruses, P. Cohen and S. van Heyningen, eds., Elsevier, New York, pp51-105 (1982).

As another aspect there is disclosed a method for treating cancers characterized by an over-expression of the c-fms proto- oncogene/M-CSF receptor gene. This method involves regionally administering to the in vivo site of such a cancer, the composition of the invention, or, alternatively, administering the composition in an ex vivo purging treatment of a mixture of cells. The composition acts by attaching to the c-fms protein on the carcinoma and delivering the toxin through the cell membrane, where the

toxin destroys the cell. Among such receptor over-expressing cancers are acute myelocytic leukemia, ovarian carcinoma, lung carcinoma, and those recited above.

Other aspects and advantages of the present invention will be apparent upon consideration of the following detailed description of the invention, including illustrative examples of the practice thereof.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates a DNA and amino acid sequence for an M-CSF polypeptide.

#### DETAILED DESCRIPTION OF THE INVENTION

The therapeutic composition of the invention is a ~~conjugate of M-CSF which is capable of binding to the c-fms~~ proto-oncogene/M-CSF receptor gene product on certain cancer cells, and a cytotoxic agent capable of being transported through the cell membrane and acting in the cytoplasm to destroy the cell.

The M-CSF for use in the present invention may be recovered from natural sources and purified. (See e.g, UK Patent 2,016,477 and PCT published application WO86/04587). Alternatively, the M-CSF may be produced recombinantly. One possible recombinant M-CSF polypeptide useful in the present invention has been described in PCT published application WO86/04607. Another M-CSF polypeptide is described in co-pending, co-owned US patent application SN940,362 and in G. G. Wong et al, Science, 235:1504-1508 (1987). The amino acid and DNA sequence of the M-CSF described therein is presented hereto in Fig. 1. Other forms of M-CSF bearing the active site thereof may also be employed in this composition, including synthetically produced polypeptides or polypeptides modified by recombinant means.

The term "M-CSF" is herein defined as including the naturally occurring human polypeptide M-CSF and naturally-

occurring allelic variations of the polypeptide. Allelic variations are naturally-occurring base changes in the species population which may or may not result in an amino acid change in a polypeptide or protein. Additionally included in this definition are both recombinant and synthetic versions of the polypeptide M-CSF, which may contain induced modifications in the peptide and DNA sequences thereof.

For example, the M-CSF polypeptide in the composition of the present invention may be characterized by a peptide sequence the same as or substantially homologous to the amino acid sequence illustrated in Fig. 1. These sequences may be encoded by the DNA sequence depicted in Fig. 1 or sequences containing allelic variations in base or amino acid sequence or deliberately modified structures coding for polypeptides with M-CSF biological properties.

Synthetic M-CSF proteins for use in the composition of the present invention may wholly or partially duplicate continuous sequences of the amino acid residues of Fig. 1. These sequences, by virtue of sharing structural and conformational characteristics with M-CSF polypeptides, e.g., the active site of the polypeptide of Fig. 1, may also possess M-CSF biological properties. Thus synthetic or recombinant polypeptides or fragments thereof may also be employed as biological or immunological equivalents for M-CSF polypeptides in the composition and methods of the present invention.

M-CSF, as used in the present invention also includes factors encoded by sequences similar to Fig. 1, but into which modifications are naturally provided or deliberately engineered. Modifications in the peptide or sequence of M-CSF can be made by one skilled in the art using known techniques. Specific modifications of interest in the M-CSF related sequences may include the replacement of one or more of the nine cysteine residues in the coding sequence with

other amino acids. Preferably several cysteines in each sequence are replaced with another amino acid, e.g. serine, to eliminate the disulfide bridges at those points in the protein. For example, lysine at amino acid position 163 (Fig. 1) could be deleted or substituted with another amino acid in order to eliminate the sensitivity of this region of M-CSF to trypsin-like proteases. Mutagenic techniques for such replacement are well known to one skilled in the art. [See, e.g., United States patent 4,518,584.]

Other specific mutations of the sequence of M-CSF described herein involve modifications of one or more of the glycosylation sites in the sequence. The absence of glycosylation or only partial glycosylation results from amino acid substitution or deletion at one, two, three or all of the asparagine-linked glycosylation recognition sites present in the sequence of M-CSF. The asparagine-linked glycosylation recognition sites comprise tripeptide sequences which are specifically recognized by appropriate cellular glycosylation enzymes. These tripeptide sequences are either asparagine-X-threonine or asparagine-X-serine, where X is usually any amino acid. A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence. Modification and variation of the types of oligosaccharides which attach to the O or N-linked glycosylation sites can occur by production of the sequence in either mammalian, bacterial, yeast or insect cells. Such modifications in the proteins are also encompassed by the term M-CSF.

Yet further modifications of M-CSF polypeptides may employ sequences which are designed for improved pharmacokinetics, by, e.g., association with polyethylene glycol. Alternatively, the last 25 to 35 amino acids of the

mature protein can be eliminated by appropriate gene deletion techniques to provide another form of M-CSF for use in the present invention. Such a deleted M-CSF may have use in genetic fusion to a cytotoxic agent. Amino acid residues 464 to 485 comprise a potential hydrophobic membrane-penetrating region. An M-CSF molecule that contains this sequence may desirably be employed in the composition of the invention, because these residues may embed the conjugate in the cell membrane, thereby aiding in the transfer of the cytotoxic agent into the cytosol.

An exemplary DNA sequence for the production of various M-CSF peptides have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD. The cDNA sequence illustrated in Fig. 1 below in vector p3ACSF-69, included in *E. coli* HB101 has been deposited on April 16, 1986 and given accession number ATCC 67092. This deposit was made under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and the Regulations thereunder (Budapest Treaty).

The cytotoxic agent linked to the M-CSF polypeptide is preferably a toxin or chemical agent which is capable of acting in the cytoplasm. Toxins may be employed which have a translocation property to move it through the cell membrane and a cytolytic domain, which provides its killing ability. One preferable class of toxins well-suited for this composition consists of two functionally different parts, termed A and B, which are connected by a disulfide bond. The A chain portion contains the enzymatic activity that enters the cytosol and kills the cell. The B chain moiety is responsible for binding of the toxin to the cell and presumably contains a domain that aids the A chain in crossing the cell membrane. Exemplary toxins for such use include native or genetically engineered ricin, abrin, modeccin, viscumin, *Pseudomonas aeruginosa* exotoxin,



Diphtheria toxin, Cholera toxin, Shigella toxin and E. coli heat labile toxin. The toxin portion of a conjugate prepared according to the invention can consist of the cytotoxic A chain portion only, the native holotoxin, or an engineered holotoxin, i.e., a toxin lacking its lectin binding property.

Other toxins which have only a single chain (an A chain portion) may also be employed. Examples of these toxins are ribosome inactivating proteins, such as pokeweed antiviral protein and gelonin. See, L. Barbieri et al, Cancer Surveys, 1:489-520 (1982) for a more complete list of ribosome inactivating proteins.

Mutant toxins or genetically engineered toxins may also be employed. Additionally microbially produced cytotoxic agents, and other non-protein organic molecules may be used as cytotoxic agents. The M-CSF ligand can also be linked to cytotoxic drugs, such as anthracyclines, e.g., doxorubicin, daunomycin, and the vinca alkaloids, such as, vindesine, vinblastine, vincristine. Methotrexate and its derivatives may also be employed as cytotoxic agents. More effective agents are those in which many molecules (between 5 to 50) of the drug are linked to the M-CSF through a polymer carrier, e.g., dextran. Bonds linking the drug to the carrier should be cleavable by the chemical environment inside the cell.

The M-CSF and a cytotoxic agent may be linked in a variety of ways. One way of linking these components is by employing one or more standard bifunctional protein crosslinkers, such as succinimidyl 3-(2-pyridyldithio)propionate (SPDP) or succinimidyl acetylthiopropionate (SATP). These crosslinkers form stable disulfide bonds between the M-CSF and toxin, or other cytotoxic agent, and yet are capable of releasing the toxin portion of the composition inside the cell, due to cleavage of the disulfide bonds by chemicals inside the cell, e.g., intracellular glutathione. These linking methods are known to those skilled in the art. See, e.g., J. Carlsson et al,

Biochem. J., 173:723-737 (1978) and N. Fujii et al, Chem. Pharm. Bull., 33:362-367 (1985). See also, A. J. Cumber et al, Methods Enzymol., 112:207-225 (1985) for other general methods for conjugating toxins to proteins.

For example, one method according to the invention involves making a M-CSF-toxin composition, using a toxin having both A and B chain. The method involves the steps of:

(a) reacting the M-CSF with sufficient crosslinker to introduce between 1 to 6 reactive groups per molecule of M-CSF. A sufficient amount of crosslinker which can be used for this purpose is between approximately 6 to 50 moles of crosslinker per mole of M-CSF dimer.

(b) reacting a toxic protein having A and B chain subunits connected by at least one disulfide bond with a conventional reducing agent, thereby liberating the chains from each other.

(c) reacting the derivitized M-CSF of step (a) with the liberated A chain subunit of the reduced toxin; and

(d) separating from the reaction mixture conjugates comprising M-CSF linked by disulfide bonds to A chain subunits.

One exemplary growth factor/toxin conjugate is prepared by this method, modifying M-CSF with SPDP, followed by conjugation of ricin A chain toxin via a disulfide bond.

Another method for making the compositions of the present invention involves the following steps:

(a) reacting the M-CSF with sufficient crosslinker to introduce between 1 to 6 reactive groups per molecule of M-CSF;

(b) reacting the derivitized M-CSF of step (a) with a holotoxin having A and B subunits attached by at least one disulfide bond, the holotoxin being functionalized with a protein crosslinker which is preferably attached to the B subunit; and

(c) separating a conjugate formed by M-CSF becoming attached to the B subunit from free M-CSF and toxin in the reaction mixture.

Another manner of linking the components of the composition of the present invention is by a genetic fusion method. See, for example, United States Patent 4,675,382.

The compositions of the present invention containing both M-CSF and a toxin can be employed in methods for treating cancers characterized by over-expression of the c-fms proto-oncogene/M-CSF receptor gene. Among such cancers are acute myelocytic leukemia, ovarian cancer, breast cancer, lung cancer, pancreatic cancer and renal cancer. The composition of the invention operates by the targeting of the c-fms proto-oncogene by the M-CSF portion of the composition. Once attached to this receptor, the M-CSF molecule aids in transporting the cytotoxic agent through the cell membrane and into the cytosol. Inside the cell, the bonds linking the cytotoxic agent to the M-CSF are cleaved by chemicals naturally within the cell and the agent is released to kill the cancer cell.

The composition of the present invention can be administered in a variety of ways including systemically, locally or regionally. Desirably the composition is administered regionally in vivo, to the site of the carcinoma. For example, it can be administered intraperitoneally, if desired, to contain its distribution to the peritoneum for use in treating a suitable cancer, e.g., ovarian cancers. Similarly for treating lung cancers, the composition could be delivered in the form of an inhalant. If desirable, the composition may be administered subcutaneously, such as bathing effected tissue after surgical removal of a tumor e.g., for breast cancers. The composition may preferably be administered intravesically for instance into the bladder. Additionally, the composition can be employed in ex vivo applications, such as "purging" of a

mixture of cells removed from a patient, for patients having a systemic cancer which is not appropriate for regional application. The treatment of patients with acute myelocytic leukemia, for example, could involve removal of bone marrow cells from the body. These cells are then treated outside the body with the composition of the present invention to destroy a subset of these cells which are overexpressing the c-fms proto-oncogene. The "purged" cells are then reintroduced into the patient. The M-CSF/toxin composition of the invention can thereby serve as a purging agent to destroy the leukemic cells in the bone marrow of AML patients about to undergo autologous bone marrow transplantation. Other ex vivo purging treatments may also employ the composition of the invention.

The therapeutic composition for use in this invention may be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such a parenterally acceptable protein solution, having due regard to pH, isotonicity, stability and the like, is within the skill of the art.

The dosage regimen involved in treating the patient with the composition according to this invention will be determined by the attending physician considering various factors which modify the action of drugs, e.g. the condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. Additionally, the mode of administration could effect the dosage, e.g., ex vivo or in vivo. Generally, the daily regimen should be in the range of 2 to 2000 micrograms of polypeptide per kilogram of body weight.

The following examples illustrate the production of the M-CSF polypeptide and the construction of an M-CSF/toxin conjugate of the present invention.

## EXAMPLE 1

Recombinant Production of M-CSF

To express the recombinant M-CSF polypeptide by recombinant means, the DNA encoding the polypeptide is transferred into an appropriate expression vector and introduced into selected host cells by conventional genetic engineering techniques.

Mammalian cell expression vectors for production of M-CSF, such as p3ACSF-69, may be synthesized by techniques well known to those skilled in this art. The components of the vectors, e.g. replicons, selection genes, enhancers, promoters, and the like, may be obtained from natural sources or synthesized by known procedures. See, Kaufman et al, J. Mol. Biol., 159:511-521 (1982); and Kaufman, Proc. Natl. Acad. Sci., U.S.A., 82:689-693 (1985). Suitable cells or cell lines for the expression of these recombinant M-CSF proteins may be Chinese hamster ovary cells (CHO), monkey COS-1 cells or CV-1 cells. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Gething and Sambrook, Nature, 293:620-625 (1981), or alternatively, Kaufman et al, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley et al, U.S. Patent 4,419,446. Other exemplary mammalian host cells include particularly primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from in vitro culture of primary tissue, as well as primary explants, are also suitable. Candidate cells need not be genotypically deficient in the selection gene so long as the selection gene is dominantly acting. For stable integration of the vector DNA, and for subsequent amplification of the integrated vector DNA, both by conventional methods, CHO cells may be employed. Other suitable mammalian cell lines include but are not limited to, HeLa, mouse L-929 cells, 3T3 lines

derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines.

Stable transformants are then screened for expression of the product by standard immunological or enzymatic assays. The presence of the DNA encoding the variant proteins may be detected by standard procedures such as Southern blotting. Transient expression of the DNA encoding the variants during the several days after introduction of the expression vector DNA into suitable host cells such as COS-1 monkey cells is measured without selection by activity or immunologic assay of the proteins in the culture medium. The transformation of these vectors into appropriate host cells can result in expression of the M-CSF.

Similarly, one skilled in the art could manipulate the sequence of Fig. 1 by eliminating or replacing the mammalian regulatory sequences flanking the coding sequence with bacterial sequences to create bacterial vectors for intracellular or extracellular expression of M-CSF by bacterial cells. The DNA encoding the factor may be further modified to contain different codons for bacterial expression as is known in the art. Preferably the sequence is operatively linked in-frame to a nucleotide sequence encoding a secretory leader polypeptide permitting bacterial expression, secretion and processing of the mature variant protein, also as is known in the art. The compounds expressed in bacterial host cells may then be recovered, purified, and/or characterized with respect to physicochemical, biochemical and/or clinical parameters, all by known methods. For example, the M-CSF coding sequence could be inserted into a known bacterial vector using procedures such as described in T. Taniguchi et al., Proc. Natl. Acad. Sci. USA, 77:5230-5233 (1980). This exemplary bacterial vector could then be transformed into bacterial host cells and the factor expressed thereby. The various strains of E. coli (e.g., HB101, MC1061) are well-known as

host cells in the field of biotechnology. Various strains of B. subtilis, Pseudomonas, other bacilli and the like may also be employed in this method. For a strategy for producing extracellular expression of such factors in bacterial cells, see, e.g. European patent application EPA 177,343.

Similar manipulations can be performed for the construction of an insect vector [See, e.g., procedures described in published European patent application 155,476] for expression in insect cells. See, e.g. Miller et al, Genetic Engineering, 8:277-298 (Plenum Press 1986) and references cited therein.

A yeast vector could also be constructed employing yeast regulatory sequences for intracellular or extracellular expression of M-CSF by yeast cells. Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides useful in the invention. [See, e.g., procedures described in published PCT application WO 86 00639 and European patent application EP 123,289.]

#### EXAMPLE 2

##### An M-CSF Toxin Conjugate

For construction of an M-CSF toxin conjugate according to the invention, the growth factor M-CSF was produced in mammalian cells as described in pending U. S. patent SN940,362, the disclosures of which are incorporated by reference herein, and G. G. Wong et al, Science, 235 supra. M-CSF (5 mg, 55 nmoles) in 0.1M NaHCO<sub>3</sub> (20 ml) was reacted with a 20-fold molar excess of SPDP in ethanol. The reaction was allowed to proceed for five hours at 4 degrees Celsius to introduce approximately four to six sulfhydryl groups per molecule of M-CSF dimer. After removal of excess SPDP the derivatized growth factor was reacted with ricin A (15 mg, 500 nmoles), obtained from a commercial source, in 50mM NaH<sub>2</sub>PO<sub>4</sub> p. 117.5/0.1M NaCl. The disulfide bond was allowed

to form overnight at 4 degrees Celsius. The resulting M-CSF-ricin A chain conjugate was separated from excess ricin A chain by gel filtration on a Sepherogel<sup>TM</sup> TSK-3000 high pressure liquid chromatography column to give a mixture of conjugate and M-CSF (7.5 mg). After two passages through a column of Blue Sepharose developed with a gradient of NaCl, as described by P. P. Knowles and P. E. Thorpe, Anal. Biochem., 160: 440-443 (1987), the conjugate (720 mg) was obtained in a form free of M-CSF and consisted mainly of a species with one ricin A chain per M-CSF dimer.

#### EXAMPLE 3

##### In Vitro Cytotoxicity of M-CSF Toxin Conjugate

A level of toxicity and specificity for the M-CSF/ricin A chain conjugate was determined in a standard soft agar clonogenic assay in a manner similar to that described by Strong et al, Blood, 65: 627-635 (1985) with the NIH 3T3 and NIH 3T3-c-fms cell lines. The latter line which has been described by M. F. Roussel et al, Nature, 325: 549-552 (1987), is M-CSF receptor positive. Each cell line was mixed with either conjugate or medium without conjugate (control) in agarose and thin layered into Petri dishes. After incubation at 37°C in standard CO<sub>2</sub> atmosphere for a period of 14 days, the number of colonies in each dish was counted visually. The NIH 3T3-c-fms cells control dishes which did not receive the conjugate showed 103 colonies per dish while the same cells treated with conjugate at a concentration of  $4 \times 10^{-8}M$  gave only 3 colonies. The NIH 3T3 cells, treated with conjugate and untreated control cells mixed with medium gave 76 and 78 colonies per dish, respectively.

#### EXAMPLE 4

##### Ex Vivo Assay of M-CSF Toxin Conjugate

The efficacy of the M-CSF/ricin A chain conjugate for ex vivo bone marrow purging is tested in a manner analogous to



that described by Strong et al, supra. M1 myeloid leukemic cells ( $10^3$ ) which may be obtained from the American Type Culture Collection, Rockville, Maryland, (ATCC TIB 192) are added to murine bone marrow cells ( $10^5$ ) and then treated with the M-CSF/ricin A conjugate in the  $10^{-7}$  -  $10^{-12}$ M range for approximately 4 hours at 37C. The percent survival of the leukemic cells as well as the monopotent and pluripotent bone marrow progenitor cells is determined with a standard colony formation assay, T.R. Bradley and D. Metcalf, Aust. J. Exp. Biol. Med. Sci., 44: 287 (1966) to measure the efficacy and specificity, respectively.

Numerous modifications may be made by one skilled in the art to the methods and components of the present invention in view of the disclosure herein. Such modifications are believed to be encompassed in the appended claims.

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International Application No: PCT/

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## MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page \_\_\_\_\_, line \_\_\_\_\_ of the description:

## A. IDENTIFICATION OF DEPOSIT:

Further deposits are identified on an additional sheet ☐.

Name of depositary institution:

American Type Culture Collection

Address of depositary institution (including postal code and country):

12301 Parklawn Drive  
Rockville, Maryland 20852 USA

Name of Deposit	ATCC No.	Referred to on page/line	Date of Deposit
p3ACSF-69	67092	6/15	16 April 1986

## C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE: (if the indications are not for all designated States)

## D. SEPARATE FURNISHING OF INDICATIONS: (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later: (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☒ This sheet was received with the international application when filed (to be checked by the receiving Office)

*Theresa L. Smith*  
(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau is:

\_\_\_\_\_  
(Authorized Officer)

## WHAT IS CLAIMED IS:

1. A therapeutic composition for treating carcinoma characterized by over-expression of the c-fms proto-oncogene/M-CSF receptor gene comprising a M-CSF polypeptide conjugated to a cytotoxic agent and pharmaceutical carrier therefor.
2. The composition according to Claim 1, wherein said cytotoxic agent is a toxin selected from the group comprising double-chain ricin, ricin A chain, abrin, abrin A chain, modeccin and modeccin A chain, Pseudomonas aeruginosa exotoxin, Cholera toxin, Shigella toxin, E. coli heat labile toxin and Diphtheria toxin, mutant toxins thereof, and recombinant versions thereof.
3. The composition according to claim 1 wherein said cytotoxic agent is selected from the group consisting of ribosome-inactivating proteins, pokeweed antiviral protein and gelonin, mutant toxins thereof, and recombinant versions thereof.
4. The composition according to claim 1 wherein said cytotoxic agent is selected from the group consisting of anthracyclines, doxorubicin, daunomycin, vinca alkaloids, vindesine, vinblastine, vincristine, methotrexate and derivatives thereof.
5. The composition according to claim 1 where said M-CSF polypeptide is conjugated to said cytotoxic agent by a heterofunctional protein cross linking agent.
6. The composition according to claim 5 where said cross linking agent is selected from the group consisting of succinimidyl 3-(2-pyridyldithio)propionate) or succinimidyl

acetylthiopropionate.

7. The composition according to claim 1 comprising M-CSF' conjugated through SPDP to a full ricin molecule.

8. A method for treating cancers characterized by an overexpression of the c-fms proto-oncogene/M-CSF receptor protein, comprising regionally administering in vivo to the site of said cancer a composition comprising M-CSF linked to a cytotoxic agent capable of crossing the membrane of the cell bearing said receptor and entering and killing the cell.

9. A method for treating cancers characterized by an overexpression of the c-fms proto-oncogene/M-CSF receptor protein, comprising ex vivo purging of a mixture of cells removed from a patient, said mixture containing said cancer cells, with a composition comprising M-CSF linked to a cytotoxic agent capable of crossing the membrane of the cell bearing said receptor and entering and killing the cancer cells.

10. A composition for treating carcinoma characterized by over-expression of c-fms proto-oncogene/M-CSF receptor gene comprising a monoclonal antibody to c-fms gene product/M-CSF receptor said monoclonal antibody conjugated to a cytotoxic agent and pharmaceutical carrier therefor.

Figure 1

10 20 30 40 50 60 70  
OCTGGGTCT CTOGGGOGCA GAGGOGCTCT COGCATOOCA GCACAGGGT GGGGGOCTOG GOOGGGGOGC

80 90 100 110 120 130 140  
CCACTOOGCA GCAGOCAGOG AGOGAGOGAG OGAGOGAGGG OGGGOGAOGC GOOGGGGOGG GAOOCAGCTG

(-32) 160 175 190  
OOOCT ATG ACC GCG OCG GGC GOC GOC GGG OGC TGC OCT OOC AOG ACA TGG CTG  
MET Thr Ala Pro Gly Ala Ala Gly Arg Cys Pro Pro Thr Thr Trp Leu

205 220 235 (1)  
GGC TOC CTG CTG TTG TTG GTC TGT CTC CTG GCG AGC AGG AGT ATC AOC GAG GAG  
Gly Ser Leu Leu Leu Val Cys Leu Leu Ala Ser Arg Ser Ile Thr Glu Glu

250 265 280 295  
CTG TOG GAG TAC TGT AGC CAC ATG ATT GGG AGT GGA CAC CTG CAG TCT CTG CAG  
Val Ser Glu Tyr Cys Ser His MET Ile Gly Ser Gly His Leu Gln Ser Leu Gln

310 325 340 355  
OGG CTG ATT GAC AGT CAG ATG CAG AOC TOG TGC CAA ATT ACA TTT GAG TTT GTA  
Arg Leu Ile Asp Ser Gln MET Glu Thr Ser Cys Gln Ile Thr Phe Glu Phe Val

370 385 400  
GAC CAG GAA CAG TTG AAA GAT OCA GTG TGC TAC CTT AAG AAG GCA TTT CTC CTG  
Asp Gln Glu Gln Leu Lys Asp Pro Val Cys Tyr Leu Lys Lys Ala Phe Leu Leu

415 430 445 460  
GTA CAA GAC ATA ATG GAG GAC AOC ATG OGC TTC AGA GAT AAC AOC OOC AAT GOC  
Val Gln Asp Ile MET Glu Asp Thr MET Arg Phe Arg Asp Asn Thr Pro Asn Ala

475 490 505  
ATC GOC ATT GTG CAG CTG CAG GAA CTC TCT TTG AGG CTG AAG AGC TGC TTC AOC  
Ile Ala Ile Val Gln Leu Gln Glu Leu Ser Leu Arg Leu Lys Ser Cys Phe Thr

520 535 550 565  
AAG GAT TAT GAA GAG CAT GAC AAG GOC TGC GTC OGA ACT TTC TAT GAG ACA OCT  
Lys Asp Tyr Glu Glu His Asp Lys Ala Cys Val Arg Thr Phe Tyr Glu Thr Pro

580 595 (122) 610 625  
CTC CAG TTG CTG GAG AAG GTC AAG AAT GTC TTT AAT GAA ACA AAG AAT CTC CTT  
Leu Gln Leu Leu Glu Lys Val Lys Asn Val Phe Asn Glu Thr Lys Asn Leu Leu

640 655 670  
GAC AAG GAC TGG AAT ATT TTC AGC AAG AAC TGC AAC AAC AGC TTT GCT GAA TGC  
Asp Lys Asp Trp Asn Ile Phe Ser Lys Asn Cys Asn Asn Ser Phe Ala Glu Cys

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Figure 1 (Con't)

685	700	715	730
TCC AGC CAA GAT GTG GTG ACC AAG OCT GAT TGC AAC TGC CTG TAC CCC AAA GGC			
Ser Ser Gln Asp Val Val Thr Lys Pro Asp Cys Asn Cys Leu Tyr Pro Lys Ala			
745	760	775	
ATC OCT AGC AGT GAC CCG GGC TCT GTC TOC OCT CAT CAG CCC CTC GGC CCC TOC			
Ile Pro Ser Ser Asp Pro Ala Ser Val Ser Pro His Gln Pro Leu Ala Pro Ser			
790	805(189)	820	835
ATG GGC OCT GTG GCT GGC TTG ACC TGG GAG GAC TCT GAG GGA ACT GAG GGC AGC			
MET Ala Pro Val Ala Gly Leu Thr Trp Glu Asp Ser Glu Gly Thr Glu Gly Ser			
850	865	880	895
TOC CTC TTG OCT GGT GAG CAG CCC CTG CAC ACA GTG GAT CCA GGC AGT GGC AAG			
Ser Leu Leu Pro Gly Glu Gln Pro Leu His Thr Val Asp Pro Gly Ser Ala Lys			
910	925	940	
CAG CCG CCA CCC AGG AGC ACC TGC CAG AGC TTT GAG CCG CCA GAG ACC CCA GTT			
Gln Arg Pro Pro Arg Ser Thr Cys Gln Ser Phe Glu Pro Pro Glu Thr Pro Val			
955	970	985	1000
GTC AAG GAC AGC ACC ATC GGT GGC TCA CCA CAG OCT CCG CCC TCT GTC GGC GGC			
Val Lys Asp Ser Thr Ile Gly Gly Ser Pro Gln Pro Arg Pro Ser Val Gly Ala			
1015	1030	1045	
TTC AAC CCC GGG ATG GAG GAT ATT CTT GAC TCT GCA ATG GGC ACT AAT TGG GTC			
Phe Asn Pro Gly MET Glu Asp Ile Leu Asp Ser Ala MET Gly Thr Asn Trp Val			
1060	1075	1090	1105
OCA GAA GAA GGC TCT GGA GAG GGC AGT GAG ATT CCC GTA CCC CAA GGC ACA GAG			
Pro Glu Glu Ala Ser Gly Glu Ala Ser Glu Ile Pro Val Pro Gln Gly Thr Glu			
1120	1135	1150	1165
CTT TOC CCC TOC AGG CCA GGA GGG GGC AGC ATG CAG ACA GAG CCC GGC AGA CCC			
Leu Ser Pro Ser Arg Pro Gly Gly Gly Ser MET Gln Thr Glu Pro Ala Arg Pro			
1180	1195	1210	
AGC AAC TTC CTC TCA GCA TCT TCT CCA CTC OCT GCA TCA GCA AAG GGC CAA CAG			
Ser Asn Phe Leu Ser Ala Ser Ser Pro Leu Pro Ala Ser Ala Lys Gly Gln Gln			
1225	1240	1255	1270
CCG GCA GAT GTA ACT GGT ACA GGC TTG CCC AGG GTG GGC CCC GTG AGG CCC ACT			
Pro Ala Asp Val Thr Gly Thr Ala Leu Pro Arg Val Gly Pro Val Arg Pro Thr			
1285	1300	1315	
GGC CAG GAC TGG AAT CAC ACC CCC CAG AAG ACA GAC CAT CCA TCT GGC CTG CTC			
Gly Gln Asp Trp Asn His Thr Pro Gln Lys Thr Asp His Pro Ser Ala Leu Leu			

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Figure 1 (Con't)

1330                      1345                      1360                      1375  
 AGA GAC CCC CCG GAG CCA GGC TCT CCC AGG ATC TCA TCA CTG CCG CCC CAG GGC  
 Arg Asp Pro Pro Glu Pro Gly Ser Pro Arg Ile Ser Ser Leu Arg Pro Gln Gly

1390                      1405                      1420                      1435  
 CTC AGC AAC CCC TCC ACC CTC TCT GCT CAG CCA CAG CTT TCC AGA AGC CAC TCC  
 Leu Ser Asn Pro Ser Thr Leu Ser Ala Gln Pro Gln Leu Ser Arg Ser His Ser

1450                      1465                      1480  
 TCG GGC AGC GTG CTG CCC CTT GGG GAG CTG GAG GGC AGG AGG AGC ACC AGG GAT  
 Ser Gly Ser Val Leu Pro Leu Gly Glu Leu Glu Gly Arg Arg Ser Thr Arg Asp

1495                      1510                      1525                      1540  
 CCG AGG AGC CCC GCA GAG CCA GAA GGA GGA CCA GCA AGT GAA GGG GCA GGC AGG  
 Arg Arg Ser Pro Ala Glu Pro Glu Gly Gly Pro Ala Ser Glu Gly Ala Ala Arg

1555                      1570                      1585  
 CCC CTG CCC CGT TTT AAC TCC GTT OCT TTG ACT GAC ACA GGC CAT GAG AGG CAG  
 Pro Leu Pro Arg Phe Asn Ser Val Pro Leu Thr Asp Thr Gly His Glu Arg Gln

1600                      1615                      1630                      1645  
 TCC GAG GGA TCC TCC AGC CCG CAG CTC CAG GAG TCT GTC TTC CAC CTG CTG GTG  
 Ser Glu Gly Ser Ser Ser Pro Gln Leu Gln Glu Ser Val Phe His Leu Leu Val

1660                      1675                      1690                      1705  
 CCC AGT GTC ATC CTG GTC TTG CTG GCT GTC GGA GGC CTC TTG TTC TAC AGG TCG  
 Pro Ser Val Ile Leu Val Leu Leu Ala Val Gly Gly Leu Leu Phe Tyr Arg Trp

1720                      1735                      1750  
 AGG CCG CCG AGC CAT CAA GAG OCT CAG AGA GCG GAT TCT CCC TTG GAG CAA CCA  
 Arg Arg Arg Ser His Gln Glu Pro Gln Arg Ala Asp Ser Pro Leu Glu Gln Pro

1765                      1780                      1795                      1817  
 GAG GGC AGC CCC CTG ACT CAG GAT GAC AGA CAG GTG GAA CTG CCA GTG TAGAGGGAAT  
 Glu Gly Ser Pro Leu Thr Gln Asp Asp Arg Gln Val Glu Leu Pro Val

1827                      1837                      1847                      1857                      1867                      1877                      1887  
 TCTAAGCTGG ACGCACAGAA CAGTCTCTCC GTGGGAGGAG ACATTATGGG GCGTCCACCA CCACCCCTCC

1897                      1907                      1917                      1927                      1937                      1947                      1957  
 CTGGCCATCC TCCCTGGAATG TGGTCTGCCC TCCACAGAG CTCCTGCGCTG CCAGGACTGG ACCAGAGCAG

1967                      1977                      1987                      1997                      2007                      2017                      2027  
 CCAGGCTGGG GCGCTCTGT CTCACCCCGC AGACCCCTGA CTGAATGAGA GAGGCCAGAG GATGCTCCCC

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Figure 1 (Con't)

2037	2047	2057	2067	2077	2087	2097
ATGCTGOCAC	TATTTATTGT	GAGOOCTGGA	GGCTOOCATG	TGCTTGAGGA	AGGCTGGTGA	GOOOGGCTCA
2107	2117	2127	2137	2147	2157	2167
GGACOOCTCTT	OOCTCAGGGG	CTGCACOOCTC	CTCTCACTOC	CTTCCATGOC	GGAAOOCAGG	OCAGGGACCC
2177	2187	2197	2207	2217	2227	2237
ACCGGOCITGT	GGTTTGTGGG	AAAGCAGGGT	GGACGCTGAG	GAGTGAAAGA	ACOOCTGCAOC	CAGAGGGCOCT
2247	2257	2267	2277	2287	2297	2307
GOCTGGTIGOC	AAGGTATTOC	AGOOCTGGACA	GGCATGGAC	TGTCTOCAGA	GAGAGGAGOC	TGAAGTTTGT
2317	2327	2337	2347	2357	2367	2377
GGGGGGGGAC	AGOGTGGGOC	TGATTTTTOOG	TAAAGGTGTG	CAGOOCTGAGA	GAGGGGGAAGA	GGAGGGCOCTCT
2387	2397	2407	2417	2427	2437	2447
GGACCTGCTG	GTCTGCACTG	ACAGOOCTGAA	GGGTCTACAC	OOCTGGGCTCA	OCCTAAGTGOC	CTGTGCTGGT
2457	2467	2477	2487	2497	2507	2517
TGOCAGGGOC	AGAGGGGAGG	OCAGOOCTGC	OOCTCAGGAOC	TGOCCTGAOCT	GOCAGTGATG	CCAAGAGGGG
2527	2537	2547	2557	2567	2577	2587
GATCAAGCAC	TGGOOCTCTGC	OOCTOCTOCT	TOCAGCAOCT	GOCAGAGCTT	CTOCAGGAGG	CCAAGCAGAG
2597	2607	2617	2627	2637	2647	2657
GCTOOOCTCA	TGAAGGAAGC	CATTGCACTG	TGAACACTGT	AOCTGOCTGC	TGAACAGOOCT	GOOOOOGTCC
2667	2677	2687	2697	2707	2717	2727
ATOCATGAGC	CAGCATOOGT	OOGTCTCTCA	CTCTOCAGOC	TCTOOOCAGC	CTOCTGCACT	GAGCTGGCOCT
2737	2747	2757	2767	2777	2787	2797
CAOCAGTOGA	CTGAGGGAGC	OOCTCAGOOCT	TGAOCTCTCTC	CTGAOCTGGC	CTTTGACTOC	COGGAGTGGA
2807	2817	2827	2837	2847	2857	2867
GTGGGGTGGG	AGAAOCTOCT	GGGGOOGOCAG	OCAGAGOOOG	TCTTTAGGCT	GTTTGTGTGG	COAGGTTTC
2877	2887	2897	2907	2917	2927	2937
TGCATCTTGC	ACTTTGACAT	TOCCAAGAGG	GAAGGGACTA	GTGGGAGAGA	GCAAGGGAGG	GGAGGGCACA



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Figure 1 (Con't)

2947	2957	2967	2977	2987	2997	3007
GACAGAGAGG	CTACAGGGGG	AGCTCTGACT	GAAGATGGGC	CTTIGAAATA	TAGGTATGCA	CCTGAGGTTG
3017	3027	3037	3047	3057	3067	3077
GGGGAGGGTC	TGCACTOOCA	AACOOOCAGG	CAGTGTCCIT	TOOCTGCTGC	CGACAGGAAC	CTGGGGCTGA
3087	3097	3107	3117	3127	3137	3147
GCAGGTTATC	OCTGTCAGGA	GOOCTGGACT	GGGCTGCATC	TCAGOOOCAC	CTGCATGGTA	TCCAGCTOOC
3157	3167	3177	3187	3197	3207	3217
ATOCACCTCT	CACOOCTTCT	TOOCTOCTGAC	CTTGGTCAGC	AGTGATGAOC	TOCAACTCTC	AOOCAOOOOC
3227	3237	3247	3257	3267	3277	3287
TCTAACATCA	OCTCTAACCA	GGCAAGOCAG	GGTGGGAGAG	CAATCAGGAG	AGOCAGGOCT	CAGCTTCCAA
3297	3307	3317	3327	3337	3347	3357
TGOCTGGAGG	GOOCTOACTT	TGTGGOCAGC	CTGTGGTGGT	GGCTCTGAGG	OCTAGGCAAC	GAGOGACAGG
3367	3377	3387	3397	3407	3417	3427
GCTGOCAGTT	GOOOCTGGGT	TOOCTTGTGC	TGCTGTGTGC	CTOCTCTOCT	GOOGOOCTTT	GTOCTOOGCT
3437	3447	3457	3467	3477	3487	3497
AAGAGAOOCT	GOOCTAACCTG	GOOGCTGGGC	OOGTGACITT	TOOCTTCTTG	OOAGGAAAG	TGAGGGTGGG
3507	3517	3527	3537	3547	3557	3567
CTGGOOOCAC	CTTOOCTGTC	CTGATGOOGA	CAGCTTAGGG	AAGGGCAGTG	AACTTGCAAT	TGGGGCTTAG
3577	3587	3597	3607	3617	3627	3637
OCTTCTAGTC	ACAGOCCTCA	TATTTGATGC	TAGAAAACAC	ATATTTTAA	ATGGAAGAAA	AATAAAAAGG
3647	3657	3667	3677	3687	3697	3707
CATTOOOOC	TCATOOOOC	AOCTTAAACA	TATAATATTT	TAAAGGTCAA	AAAAGCAATC	CAAOCCACTG
3717	3727	3737	3747	3757	3767	3777
CAGAAGCTCT	TTTTGAGCAC	TGGTGGCAT	CAGAGCAGGA	GGAGOOOCAG	AGOCACCTCT	GGTGTOOOOC
3787	3797	3807	3817	3827	3837	3847
CAGGCTAOC	GCTCAGGAAC	COCTTCTGTT	CTCTGAGAAG	TCAAGAGAGG	ACATTGGCTC	ACGCACTGTG

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Figure 1 (Con't)

3857 3867 3877 3887 3897 3907 3917  
AGATTTTGT TTTATACTTG GAAGTGGTGA ATTATTTTAT ATAAAGTCAT TTAATATCT ATTAAAAA

3927 3937 3947 3957 3967 3977  
TAGGAAGCTG CTTATATATT TAATAATAAA AGAAGTGCAC AAGCTGOOGT TGAOGTAGCT OGAG

# INTERNATIONAL SEARCH REPORT

International Application No. **PCT/US88/03697**

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup> According to International Patent Classification (IPC) or to both National Classification and IPC Int. Cl <sup>4</sup> A61K 37/02, 31/705; C07K 17/06; C07H 15/24 U.S. CL 514/2, 8; 424/85.1; 530/351, 402, 403, 404, 405, 406																				
<b>II. FIELDS SEARCHED</b> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Minimum Documentation Searched <sup>7</sup></div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 25%; border: 1px solid black;">Classification System</th> <th style="border: 1px solid black;">Classification Symbols</th> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: middle;">U.S.</td> <td style="border: 1px solid black;">514/2, 8; 424/85.1; 530/351, 402, 403, 404, 405, 406</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup></div> <p>Computer Search on CAS and Dialog; Files CA, Biosis, 155, 350, 351; For: CSF and (conjugate or link or complex or couple) and (toxin or cytotoxic agent or anthracycline)</p>			Classification System	Classification Symbols	U.S.	514/2, 8; 424/85.1; 530/351, 402, 403, 404, 405, 406														
Classification System	Classification Symbols																			
U.S.	514/2, 8; 424/85.1; 530/351, 402, 403, 404, 405, 406																			
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%; border: 1px solid black;">Category <sup>10</sup></th> <th style="width: 70%; border: 1px solid black;">Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup></th> <th style="width: 20%; border: 1px solid black;">Relevant to Claim No. <sup>13</sup></th> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">Y</td> <td style="border: 1px solid black; vertical-align: top;">US A, 4,504,586, (Nicolson), March 12, 1985, See Columns 1-2</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-8</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">Y</td> <td style="border: 1px solid black; vertical-align: top;">Science, Vol. 236, Issued June 1987; "The Human Hematopoietic Colony-Stimulating Factors", (Clark), pages 1229-37, See pages 1235-36.</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-8</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">Y</td> <td style="border: 1px solid black; vertical-align: top;">Blood, Vol. 67, Issued February 1986, "The Molecular Biology and Functions of the Granulocyte - Macrophage Colony - Stimulating Factors", (Metcalf), pages 257-67, See pages 259, 262-64.</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-8</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">Y</td> <td style="border: 1px solid black; vertical-align: top;">US, A, 4,675,382, (Murphy), June 23, 1987 See Columns 1-3.</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-2, 8-9</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">Y</td> <td style="border: 1px solid black; vertical-align: top;">Pharmac. Ther, Vol. 15, Issued 1982, "Chimeric Toxins", (Olsnes), pages 355-79, See pages 355, 357-62, 366.</td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-8</td> </tr> </table>			Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>	Y	US A, 4,504,586, (Nicolson), March 12, 1985, See Columns 1-2	1-8	Y	Science, Vol. 236, Issued June 1987; "The Human Hematopoietic Colony-Stimulating Factors", (Clark), pages 1229-37, See pages 1235-36.	1-8	Y	Blood, Vol. 67, Issued February 1986, "The Molecular Biology and Functions of the Granulocyte - Macrophage Colony - Stimulating Factors", (Metcalf), pages 257-67, See pages 259, 262-64.	1-8	Y	US, A, 4,675,382, (Murphy), June 23, 1987 See Columns 1-3.	1-2, 8-9	Y	Pharmac. Ther, Vol. 15, Issued 1982, "Chimeric Toxins", (Olsnes), pages 355-79, See pages 355, 357-62, 366.	1-8
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Y	Blood, Vol. 67, Issued February 1986, "The Molecular Biology and Functions of the Granulocyte - Macrophage Colony - Stimulating Factors", (Metcalf), pages 257-67, See pages 259, 262-64.	1-8																		
Y	US, A, 4,675,382, (Murphy), June 23, 1987 See Columns 1-3.	1-2, 8-9																		
Y	Pharmac. Ther, Vol. 15, Issued 1982, "Chimeric Toxins", (Olsnes), pages 355-79, See pages 355, 357-62, 366.	1-8																		
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>10</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"Δ" document member of the same patent family</p> </div> </div>																				
<b>IV. CERTIFICATION</b> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border: 1px solid black; vertical-align: top;">         Date of the Actual Completion of the International Search   <div style="text-align: center;">24 January 1989</div>         International Searching Authority   <div style="text-align: center;">ISA/US</div> </td> <td style="width: 50%; border: 1px solid black; vertical-align: top;">         Date of Mailing of this International Search Report   <div style="text-align: center; font-size: 1.2em;">08 MAR 1989</div> <div style="text-align: center;">           Signature of Authorized Officer             Garnette D. Draper         </div> </td> </tr> </table>			Date of the Actual Completion of the International Search  <div style="text-align: center;">24 January 1989</div> International Searching Authority  <div style="text-align: center;">ISA/US</div>	Date of Mailing of this International Search Report  <div style="text-align: center; font-size: 1.2em;">08 MAR 1989</div> <div style="text-align: center;">           Signature of Authorized Officer             Garnette D. Draper         </div>																
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Exp. Clin. Cancer Res. Vol. 3, Issued 1984, "Biochemical Aspects of Antibody - Directed Delivery of Toxins and Drugs to Target Cancer Cells, (Chersi), pages 217-23.	1-8
Y	Monoclonal Antibodies '84: Biological and Clinical Applications, Issued 1985, "Antibody Carriers of Cytotoxic Agents in Cancer Therapy: A Review" (Thorpe), pages 475-506.	1-8